NITROGENOUS EXCRETION IN THE TROPICAL SEA URCHIN DIADEMA ANTILLARUM PHILIPPI

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Echinoids are considered to be predominantly ammonotelic in the excretion of their nitrogenous metabolic end products (Prosser and Brown, 1961; Nicol, 1960). Other excretory products have, however, been found in the perivisceral fluid and tissue of echinoids or in the surrounding sea water of vessels in which specimens have been enclosed. Delaunay (1931) found that considerable amounts of amino acids were excreted by the urchins *Paracentrotus* and *Strongylocentrotus,* as well as small amounts of urea, uric acid and other purines. Conheim (1901) found urea, amino nitrogen, ammonia and purine bases in the coelomic fluid of urchins. Sanzo (1907) found urea in several species and Myers (1920) found creatine, creatinine, uric acid, urea and ammonia in *Strongylocentrotus franciscanus.* Van der Heyde (1923) found only uric acid in the coelomic fluid and intestine of *Arbacia,* while Przylecki (1926) found only traces of uric acid in echinoids. Boolootian (1961) has listed the amounts of nitrogenous excretory elements in the perivisceral fluid found in a number of echinoids.

Excretion studies on echinoids have been mainly concerned with temperate and cold-water species. The determination of the excretory products and rate of excretion of *Diadema antillarum* is thus of interest in terms of the physiology of a sea urchin of widespread occurrence in the tropics.

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Methods

Experiments were performed on freshly collected specimens of 5 to 7 cm. test diameter. Specimens were placed in covered glass jars filled with a measured amount of filtered sea water, of between 2 and 4 liters. A control jar contained no urchin. Tests were run for 4 hours at approximately sea temperature (26–28° C.). At the completion of the trial the urchins were removed and the water filtered to remove faecal matter and other solids. Analyses for excretory products in the water were begun immediately.

Descending paper chromatograph techniques were used as qualitative tests for excretory products in the "excretory water" as well as in the pericisceral fluid. After electrolytic desalting (Baird & Tatlock desalting apparatus) 0.5 ml. of fluid or water was evaporated on a paper pad. This pad was then affixed with a plastic clip to the top of the paper chromatogram strip. Appropriate amounts (1–10 μg., depending on sensitivity of method) of reagent quality samples of the substances

being tested for were applied to paper pads and affixed to adjacent paper strips as standards, according to the methods of Smith (1960).

Urea was tested for by the methods of Block *et al.* (1958), using phenol with sodium hypochlorite and with acetone and dimethylaminobenzaldehyde (Smith, 1960). Uric acid and other purines were tested for by the method of Block *et al.* (1958), using diphenylcarbazone on acidified mercuric acetate in ethanol and by ultra-violet light (Smith, 1960). Creatine and creatinine were tested for by the picric acid method (Block *et al.,* 1958) and amino acids were identified with ninhydrin.

Ammonia and total nonprotein nitrogen were determined quantitively by micro-diffusion methods (Conway, 1962). Kjeldahl treatments of sea water samples prior to diffusion determinations were modified to use 200 ml. of water according to the method of Barnes (1959). Amino acid quantitative determinations were a micro-diffusion modification of the ninhydrin method of Sobel *et al.* (1945). Tests for urea by diffusion were also run according to Conway (1962), using urease tablets (British Drug Houses). Filtered sea water with and without urease were run as controls and replicate samples were tested.

TABLE I

Mean hourly production in μg. of excreted nitrogen by Diadema

Total N.P.N.	NH_3N	%NH_3N	Amino N.	% Amino N.	Number of animals	Duration exp. in hours
162	99	61	42	26	12	4
245	157	64	71	29	12	4

RESULTS

In spite of repeated attempts to distinguish them in both perivisceral fluid and in the "excretory water" of *Diadema,* urea, uric acid or other purines, creatine and creatinine were not found by the methods used. Since these methods were sensitive to a few micrograms of the detectable substances, it would appear that urea, purines and creatine are only present in very minute amounts if indeed they are nitrogenous excretory products. The amounts of urea, purines, and creatine and creatinine noted by Boolootian (1961) for other echinoids were of the same order as the amounts detectable by the chromatographic methods used here.

Amino acids, however, were found in sensible amounts, together with ammonia. The results of analyses of excreted ammonia nitrogen, nonprotein nitrogen and amino acid nitrogen in excretory water are shown in Table I.

The highest proportion of excreted nitrogen occurred as ammonia nitrogen in two series of experiments. In the first series, urchins were freshly collected, while in the second series the specimens had been previously fed for 12 hours on a diet of fish meal.

The results of analyses of samples of perivisceral fluid showed no urea, purines, creatine or creatinine. Substantial amounts of amino acids were detected on paper chromatograms, however, and ammonia nitrogen was also present. The amounts of ammonia nitrogen in the fluid were found to vary between 42 and 148 μg. per

100 ml. of fluid in freshly collected specimens. The mean content of 20 specimens was 100 μg. of ammonia nitrogen per 100 ml. of fluid. The ammonia nitrogen content of the fluid of animals which had been fed on a protein diet for 24 hours was markedly higher. Amounts of ammonia nitrogen in 16 specimens varied between 255 and 645 with a mean of 374 μg. of nitrogen per 100 ml. of fluid.

Since no specific excretory organ is known for echinoids it is of interest to compare the amounts of ammonia nitrogen found in the various tissues. The amounts of ammonia nitrogen were obtained by grinding a known weight of tissue in distilled water which was free of ammonia and subsequently determining the ammonia by diffusion (Conway, 1962).

Table II

Mean ammonia nitrogen concentrations in μg./100 gm. in various tissues of Diadema

Organ	Ammonia N. μg./100 gm.	No. specimens analyzed
Oesophagus	159	12
Caecum	195	12
Foregut	317	12
Hindgut	400	12
Rectum	155	12
Gonad	68	12
Muscle	61	12
Gills	75	12

The results of determinations of tissue ammonia contents are shown in Table II. The results show increasing concentrations of ammonia nitrogen in the gut towards the rectum. Concentrations rose from 159 μg. in the oesophagus to 400 μg. per gm. of tissue in the hind gut. Concentrations in the gills, gonads and muscle were comparatively low.

Discussion

Like most other sea urchins, *Diadema* excretes the largest proportion of its nitrogenous waste as ammonia but substantial amounts of amino acids were also excreted. The amounts of ammonia nitrogen found in the perivisceral fluid are comparable to those found in other urchins. Delaunay (1931) recorded a value of 240 μg./100 ml. in *Paracentrotus lividus* and Myers (1920) found 80 μg./100 ml. in *Strongylocentrotus franciscanus*. Unlike other urchins whose excretory physiology has been investigated, no urea, purines, creatine or creatinine have been found.

The ammonia content of the various tissues is of interest for it suggests increased excretory activity towards the distal end of the hind gut. Sections of the hind gut just preceding the rectum had more than twice the amount of tissue ammonia than in the oesophagus and caecum. Progressive increase in tissue ammonia along the gut in insects has been interpreted as denoting areas of excretory function (Lennox, 1940; Staddon, 1955). However, the degree of differences in ammonia content found in insects was far greater than occurred here in *Diadema*. The hind gut was considered to have an excretory function in echinoids by Van der Heyde (1923) and Delaunay (1931).

Summary

Diadema antillarum is ammonotelic in its excretion of nitrogenous waste products. It excretes approximately 60% of its total nonprotein nitrogen as ammonia and approximately 30% as amino acids. No urea, uric acid or other purine bases were found to be excreted. A progressive increase in tissue ammonia content in the intestine towards the rectum suggests that the hind gut has an excretory function.

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